Evaluation of Leaflet Washout in Vein Valves

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Background and Goals

Background:

- Professor Tranquillo's Lab is developing engineered tissue vein valves, composed of two leaflets that function at low pressures.
- During development, testing must be done in order to determine if vein valves are ready for implantation.
- Washout testing can be performed by putting a valve in a closed flow loop, injecting dye behind the leaflets, and cycling fluid through the flow loop while measuring the amount of time it takes for the dye to washout from behind the leaflets (Tanner, 2013).
- Washout testing is mainly used to determine if all the fluid behind the leaflets washes out at a given flow rate.
- Washout testing is also applicable because of the threat of leaflet fusion to side walls of the vein. When blood flow is stagnant for long periods the valve can remain open, leading to clotting and thus fusion to the walls

Goals:

- Determine an optimal valve design using washout testing, by creating various vein valves and conducting washout testing on each of them.
- Determine if shorter leaflets are viable and do not have a detrimental effect on the valves' efficiency.

Materials/Methods

- Engineered tissue samples were used to create vein valves.
- The tissue samples were cut into slabs that represented the leaflets, and were sewn together on the edges.
- The leaflets were then attached to a stent.
- Leaflet lengths : 16, 18 and 20 mm.
- Leaflet height: 7 mm.
- Stented tissue engineered vein valves were then placed into a pulse duplicator system with a camera to view the valve's motion.
- A dye injection device was also built and attached
- 0.2 mL of dye were injected during each test
- Windows Movie Maker was used to determine washout time, time to peak dye intensity, and injection time.

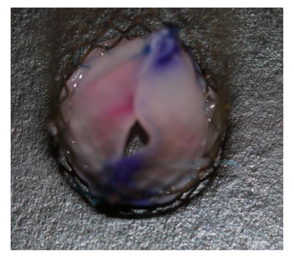


*Figure 1:*Top View of Stented Vein Valve

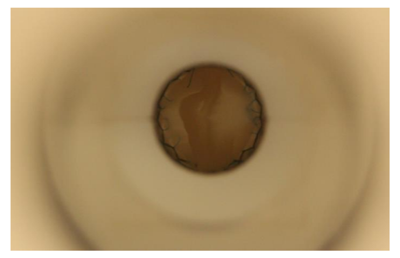


Figure 2: Front View of Stented Valve in Pulse Duplicator System

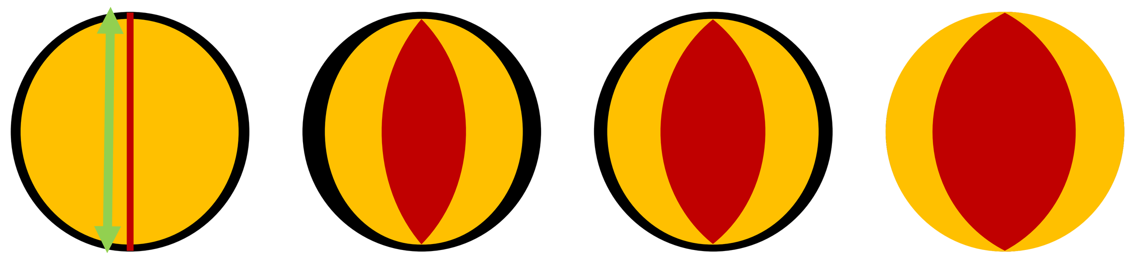


Figure 3: Front View Diagram of Different Leaflet Lengths. A Closed Valve is Shown on the Left, Increasing Leaflet Lengths Going Right

Dye Injection System

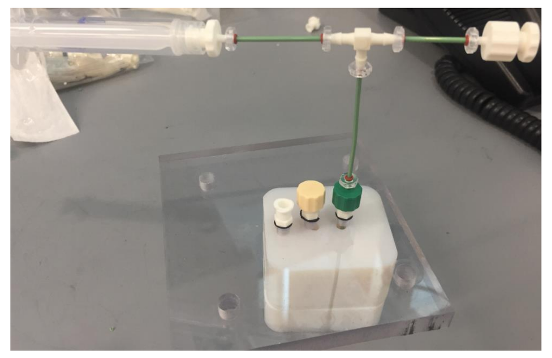


Figure 4: Dye Injection System

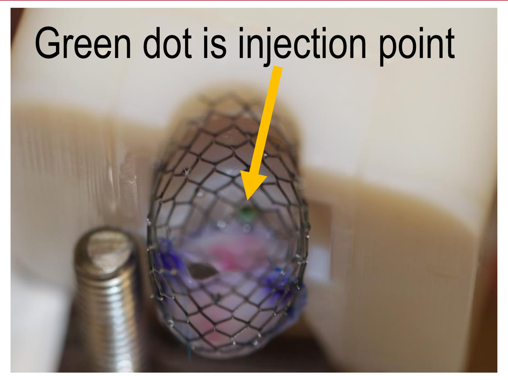


Figure 5: Stented Vein Valve in Dye Injection System

Washout Testing Methods

Method 1: Dye injection during cyclic flow

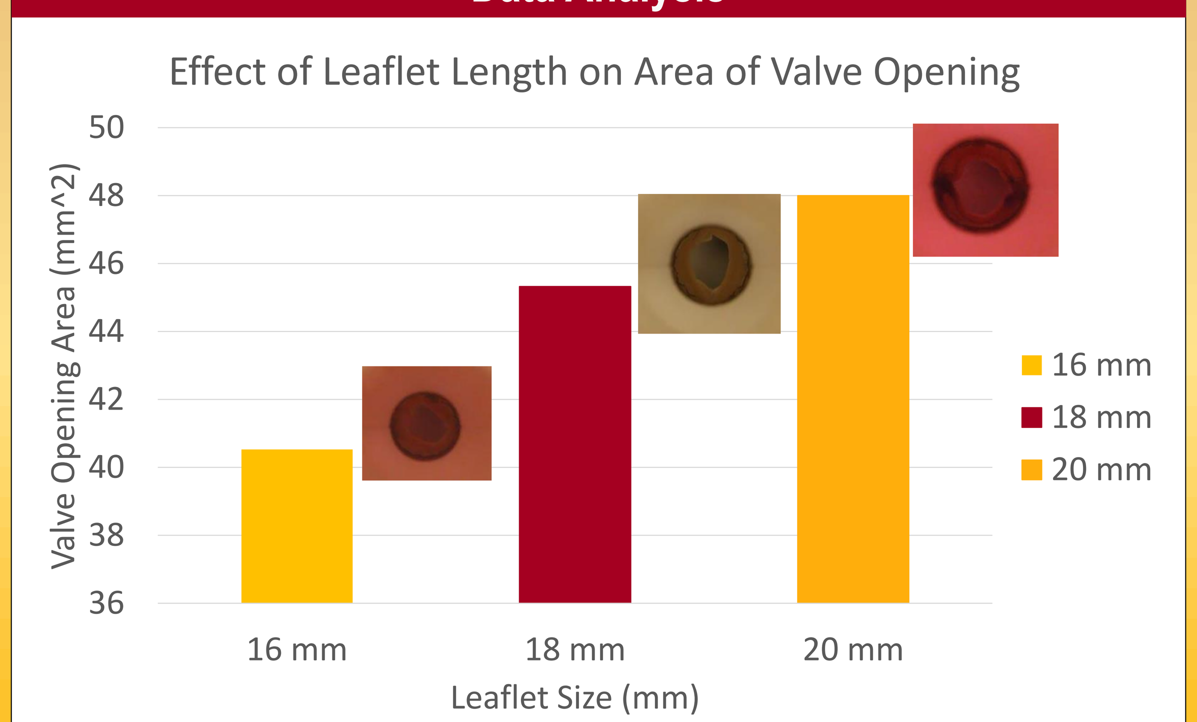
- Pulse duplicator was kept on, so the valve was opening and closing while injecting dye
- Washout time: time from when injection stopped to when the color of the chamber remained the same (the dye washed out to the fullest extent possible)
- The time to peak intensity: time from when injection stopped to when the chamber showed the most intense color (visually determined)
- The injection time: time from when injection started to when no more dye was visibly added

Method 2: Dye injection prior to cyclic flow

- Injection of the dye before the pulse duplicator was turned on
- Washout time: time from the opening of the valve to when the color in the chamber of the pulse duplicator system remained constant
- The time to peak intensity: time from the opening of the valve to when the chamber showed the most intense color

Note: Measured times were converted to cycles using time for one cycle of opening and closing of the valve.

Data Analysis

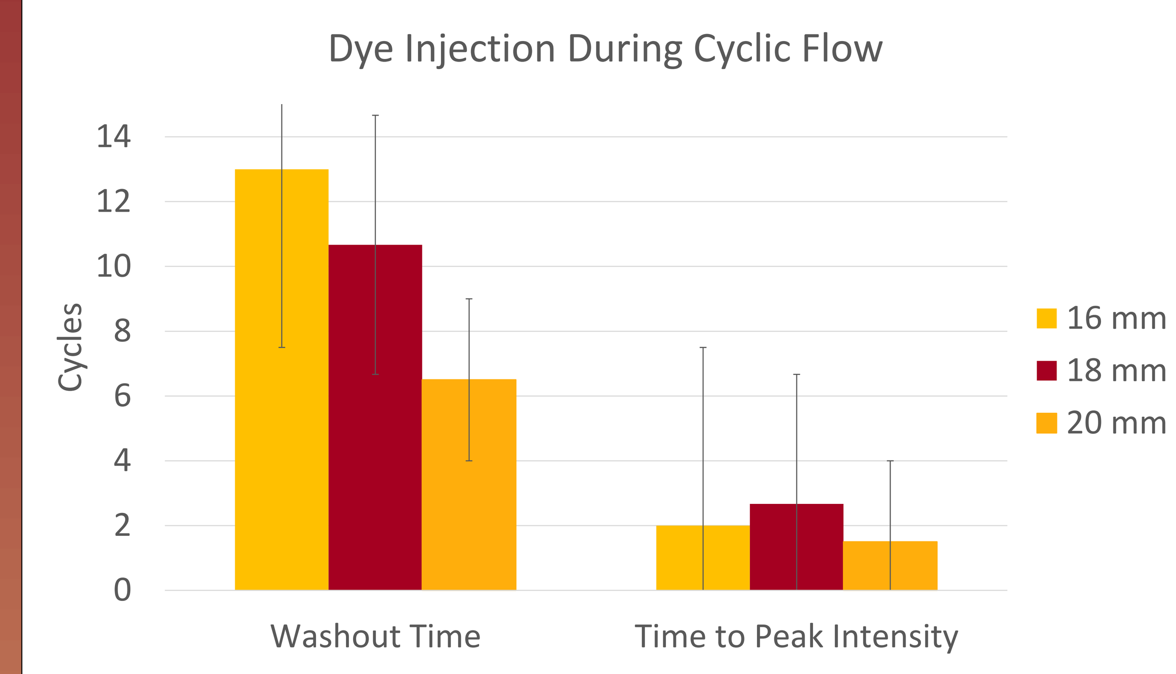


Leaflet Length (mm)	16	18	20
Area of Valve Opening (mm^2)	40.52	45.32	47.99

Figure 6: Area of Valve Opening for each Leaflet Size Tested.

- The area of the valve opening was calculated using ImageJ and the photographs that were obtained from the original setup
- Increasing leaflet size decreased how much leaflets touched the walls

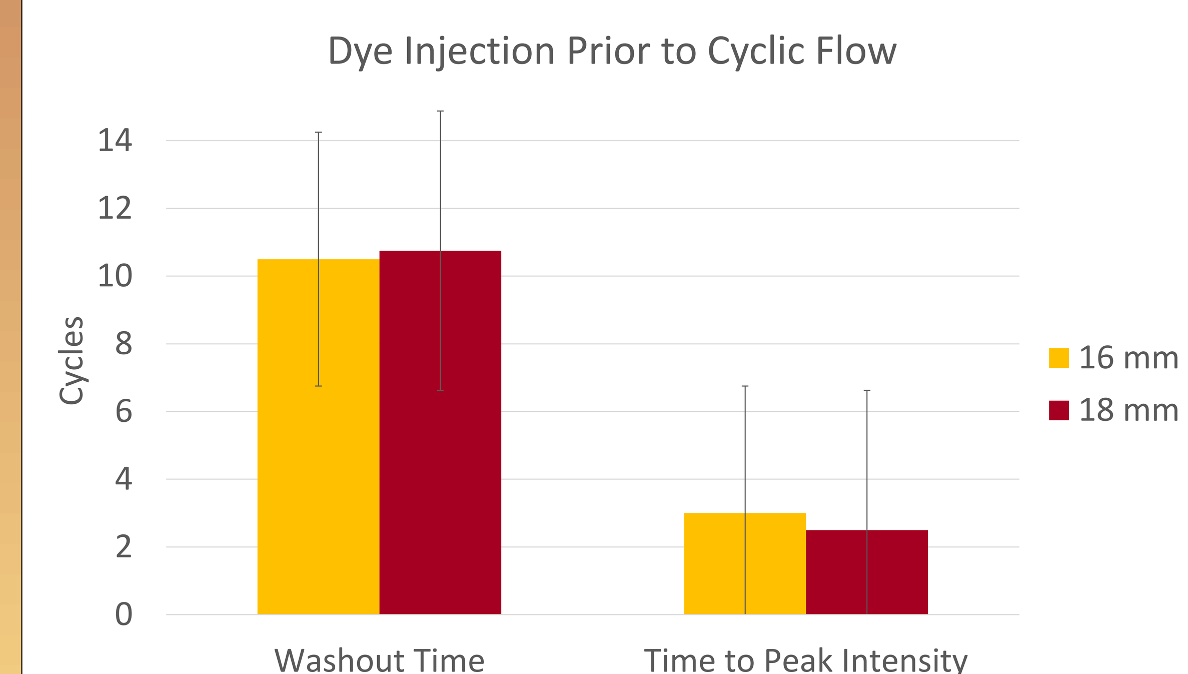
Data Analysis



Dye Injection During Cyclic Flow (cycles)

Leaflet Length	*Washout Time Avg.*	*Washout Time St Dev*	*Time to Peak Intensity Avg.*	*Time to Peak Intensity St Dev*
16 mm	13	0	2	0
18 mm	10.67	1.25	2.67	0.94
20 mm	6.5	0.5	1.5	0.5

Figure 7: Washout Time and Time to Peak Intensity for Three Leaflet Sizes using Method 1 (Dye Injection During Cyclic Flow)



Dye Injection Prior to Cyclic Flow (cycles)

Leaflet Length	*Washout Time Avg.*	*Washout Time St Dev*	*Time to Peak Intensity Avg.*	*Time to Peak Intensity St Dev*
16 mm	10.5	0.5	3	0
18 mm	10.75	0.75	2.5	0.5

Figure 8: Washout Time and Time to Peak Intensity for Three Leaflet Sizes using Method 2 (Dye Injection Prior to Cyclic Flow)

T-Tests

- There was no significant difference between the washout time and time to peak intensity between the two methods
- There was no significant difference in washout time and time to peak intensity between the different leaflet sizes
- Significance level of 0.05 was chosen

Comparing 'Dye into Continuous Flow' to 'Dye Prior'

	Washout Time	Time to Peak Intensity
p value	0.95	0.85

Comparing 16 mm to 18 mm

	Washout Time	Time to Peak Intensity
p value	0.81	0.5

Images of Flow Over Time

Flow of dye through the valves over time.

- Images collected from videos of the valves in the pulse duplicator system
- Time to peak intensity and washout time varied between trials
- Third image in Figure 8 (120 frames) was close to what the valve would have looked like at time to peak intensity
- The valve looked similar to what was seen at the end for both Figures 8 and 9 (296 frames) at the completion of washout

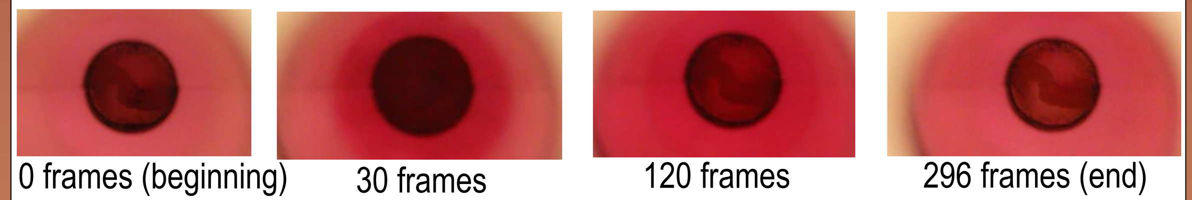


Figure 8: Images of Flow during Method 1 (Dye Injection During Cyclic Flow

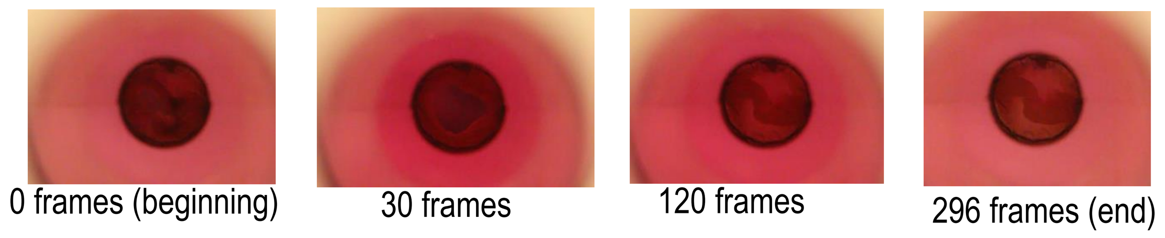


Figure 9: Images of Flow during Method 2 (Dye Injection Prior to Cyclic Flow

Conclusions

- There was not a large difference in the washout times and time to peak intensity between the different leaflet sizes.
- Smaller leaflet size does not compromise the integrity of the valve and efficiency of the valve. However, smaller leaflets prevent the leaflets from touching the walls, and thus allows for decreased risk of fusion to the walls.
- The time to peak intensity and washout times were also not found to be different between the two methods. Thus, either method can be used.
- Washout time is based more on fluid flow and stroke volume. It doesn't matter which method is used for a given leaflet size because the amount of fluid the leaflets move through the valve is the same, and the flow is held constant between methods.
- Future work could include further testing in order to confirm the results and to understand the effects of different leaflet sizes on efficiency of vein valves. A total of 10 trials were conducted in this experiment.

Acknowledgements

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References

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